Antihistamine Potentiation of Pentobarbital Anesthesia*

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The results of this investigation indicate that the common antihistamine drugs significantly prolong the sleeping time of pentobarbital anesthesia in the rat. The results of liver analyses indicate that the antihistamines do not seem to interfere with detoxication of pentobarbital. Evidence is presented to support the assumption that the antihistamines cause an increase in the rate of entrance of pentobarbital into the brain. The results also indicate that there is a required threshold concentration of pentobarbital in the blood necessary before the antihistamines produce an altered response. The mechanism by which the antihistamines prolong the sleeping time of pentobarbital is apparently unrelated to their common side actions.

THE COMMON SIDE ACTIONS of the antihistamine drugs have been extensively summarized in numerous reports (1–8). Both depression and stimulation of the central nervous system have been observed after administration of the antihistamines (9). The most extensive report on the toxic actions of the antihistamines was by Wyngaarden and Seevers (10). In an attempt to estimate the potential central nervous system depressant action of the antihistamines Winter suggested the administration of a drug of known sedative action and observing the effect of an antihistamine superimposed upon it. He subsequently reported a potentiating effect upon Evipal® (hexobarbital) anesthesia (40–100 mg./Kg.) by using Benadryl®, Pyribenzamine® and Neo-Antergan® (10–20 mg./Kg.) prior to the injection of barbiturate (11). This same phenomenon of potentiation of barbiturate anesthesia was reported by Heinrich (12). However, Cronheim and Ehrlich (13) reported an absence of any potentiating effect using four antihistamines related to Pyribenzamine®. The latest evidence to support the earlier work has been reported by Ambrus, *et al.* (14), who found certain antihistamines potentiated the effect of Evipal® in mice. It is the purpose of this paper to report results obtained by the use of five common antihistaminics in an attempt to demonstrate potentiation of pentobarbital narcosis in rats, and to offer a possible explanation for such a phenomenon.

EXPERIMENTAL

Potentiation.—Wistar strain rats obtained from Carworth farms were used throughout the investigation. In most experiments they were employed in groups of twelve, selected to make the average weight of the control group correspond as closely as possible to the average weight of the experimental animals. In all experiments the rats were fasted for eighteen to twenty-four hours prior to use. The antihistamine drug solutions were prepared in distilled water so as to contain 10 mg./cc. and were not utilized if older than twenty-four hours. The pentobarbital used in this investigation was Abbott's Veterinary Nembutal®, containing 60 mg./cc. Where the effect of the antihistamine upon the recovery time of rats from narcosis induced with pentobarbital was to be observed, the rats received 10 mg./Kg. of the antihistamine subcutaneously, followed in thirty minutes by 60 mg./Kg. of pentobarbital administered intraperitoneally. The time from the loss of the righting reflex to return of the reflex was taken as the duration of sleep. The righting reflex normally returns after the following signs occur: yawning, stretching, movements of the vibrissae, urination, and erection of the tail. In some cases the rats served as their own controls and when such procedure was adopted at least four days was allowed to lapse between experiments. Diphenhydramine hydrochloride (Benadryl®), β-(4-bromobenzhydryloxy)-ethyl-dimethylamine hydrochloride (Ambodryl®), tripelennamine hydrochloride (Pyribenzamine®), phenindamine tartrate (Thephorin®), and chlorphenamine maleate (Chlor-Trimeton®) were used and the doses always refer to the salts of these compounds. Ambodryl® is a bromine analog of Benadryl® and is said to be less depressant in its side action when employed clinically.

Tissue Studies.—All tissues were analyzed for pentobarbital by the method of Goldbaum (15). In the liver analyses twelve rats were used for controls and twelve rats for experimentals. In the case of the brain analyses fifteen rats were used in the experimental study and twelve served as controls. Three rats were used for each time period and analyses were made on individual livers and brains so that three separate readings could be compared. In the experiments comprising the tissue studies the rats received 30 mg./Kg. of pentobarbital instead of the usual 60 mg./Kg. The animals were sacrificed at specified time intervals and tissues immediately removed and placed in vessels at a temperature of zero degrees centigrade. They were analyzed for pentobarbital no longer than one hour later.

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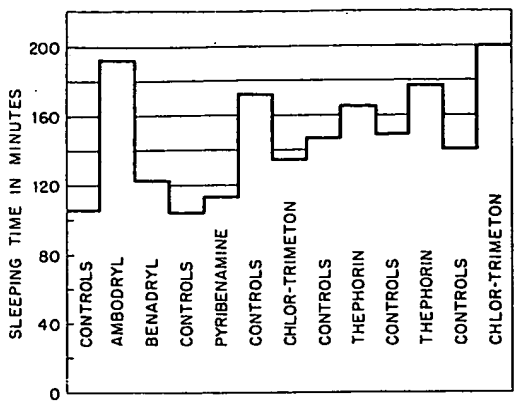

Fig. 1.—Effect of antihistamines on pentobarbital anesthesia using separate control groups.

STATISTICAL

	1	2	3	4	5	6	7
$S_{\bar{x}}$	34.23	1.03	3.16	8.25	16.4	10.5	12.
t	0.29	1.65	1.21	1.42	1.18	2.07	3.9
p	>0.5	0.12	0.25	<0.2	<0.3	<0.1	<0.1

RESULTS AND DISCUSSION

Synergism Between Antihistamines and Pentobarbital Using Separate Control Groups.—In a series of six experiments the effects of Benadryl®, Ambodryl®, Pyribenzamine®, Chlor-Trimeton®, and Thephorin® on the sedative action of pentobarbital was studied. In Fig. 1 the sleeping times of the rats given both antihistamine and pentobarbital can be compared to the control group which received only pentobarbital. In all cases but one (Chlor-Trimeton®) the experimental animals slept longer than the controls. It should be pointed out however, that the controls in this particular case had received an antihistamine agent five days earlier.

TABLE I.—EFFECT OF ANTIHISTAMINES ON SLEEPING TIME OF PENTOBARBITAL IN RATS SERVING AS OWN CONTROLS

	Sleeping Times in Minutes					
	P[a]	H[b]	P	H	P	H
Controls	184	185	152	132	135	120
Benadryl®	116	145	145	190	75	121
Ambodryl®	138	168	171	224	101	147
Pyribenzamine®	136	161	188	173	93	97
Thephorin®	108	139	123	151	84	81
Chlor-Trimeton®	108	165	141	170	92	103

[a] P—pentobarbital alone.
[b] H—pentobarbital plus antihistamine.
The controls received pentobarbital alone throughout the experiment.

Since it is generally agreed among various investigators that the antihistamines are released slowly from the body and hardly eliminated within five days (16), it is a possibility that the synergistic action was still present even after five days.

Synergism Between Antihistamines and Pentobarbital in Rats Serving as Their Own Controls.—Since the sleeping times of two groups of rats in the same weight range under pentobarbital anesthesia may vary considerably, it was deemed necessary to observe what happens when rats serve as their own controls and receive pentobarbital alone and in combination with antihistamines on alternate occasions.

Five groups of rats were selected to be used with the antihistamines and another group to serve as controls. The control group was run to observe what effect the pentobarbital alone produced on successive administrations. The average weights of the five groups were within 5 Gm. of each other. The sleeping times are recorded in Table I. It will be noted in all cases but two that the sleeping times of rats receiving antihistamines plus pentobarbital were longer than those of rats receiving pentobarbital alone.

The control group showed an almost progressive decline in sleeping times. In Table II the statistical evaluation for all the groups is shown. The probability of the results occurring by chance is less than when the sleeping times were compared to those of separate control groups.

Determination Whether the Antihistamines Cause a True Potentiation of the Pentobarbital.—In approaching the mechanism of the prolongation of sleeping times of pentobarbital by the antihistamines an effort was made to see if the response to a lower than ED_{25} could be increased. Each rat of a group of fifteen averaging 108 Gm. in weight, was given a dose of 20 mg./Kg. of pentobarbital. Four out of the fifteen rats lost the righting reflex. After an interval of four days, the same group was given the same dose of pentobarbital preceded this time by 10 mg./Kg. of Ambodryl®. Again only four rats lost the righting reflex. These results suggest an action other than true potentiation; at least with this dose of pentobarbital the modification of response is not apparent.

In an effort to gain more facts which might shed light upon this altered response to pentobarbital another experiment was carried out in which fifteen rats averaging 130 Gm. in weight were each given 22.5 mg./Kg. of pentobarbital. Six of the fifteen went to sleep for three to twenty-eight minutes. Five days later the same rats were given 10 mg./Kg. of Ambodryl® followed in a half hour by 22.5 mg./Kg. of pentobarbital and in this case twelve of the

TABLE II. SIGNIFICANCE OF RESULTS

Trial	1		2		3	
	t value	p[a]	t value	p	t value	p
Benadryl®	2.09	0.08	1.35	0.25	2.62	0.04
Ambodryl®	2.16	0.08	2.33	0.06	3.45	0.015
Pyribenzamine®	1.50	0.19	..	..	..	..
Theophorin®	1.86	0.08	2.38	0.06	..	..
Chlor-Trimeton®	3.42	0.015	1.57	0.18	1.1	0.3

[a] p—probability.

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fifteen rats went to sleep and slept for eleven to forty-five minutes. From an examination of these results it would seem that the dose of pentobarbital is an important factor in determining whether or not a previous dose of antihistaminic will increase the response to the pentobarbital. In raising the dose from 20 to 22.5 mg./Kg. a 50% difference resulted between the control group and the experimental group in the number of animals losing the righting reflex. This difference in the dose would suggest that a certain threshold level of circulating pentobarbital was necessary before any significant modification of response could be produced. These facts would weigh heavily in favor of the increased permeability of the brain as the cause of the increased sleeping time. Thus, in the first instance the amount of circulating unbound pentobarbital was of such a level that the concentration in the brain necessary to produce anesthesia was never attained.

The Antihistamines Cause a Mobilization of Pentobarbital in the Brain.—An experiment to ascertain whether the antihistamines increase the rate of entrance of pentobarbital into the brain was performed. Twelve rats were used and each was injected with 60 mg./Kg. of pentobarbital. Ambodryl® (10 mg./Kg.) was given intraperitoneally as each rat regained the righting reflex. Six of the twelve animals relost the righting reflex and slept for eighteen to seventy-five minutes. These results also would seem to favor the theory of increased penetration of the brain by pentobarbital.

The following experiments were undertaken to ascertain some of the chemical aspects of the phenomenon with which we are concerned. It was agreed that if it could be shown that the antihistamines interfere with the normal mechanisms of the tissues in dealing with pentobarbital, some explanation of the prolonged sleeping times might be forthcoming. Since the liver and the brain are the principal organs with which we are concerned when dealing with the barbiturates, it was decided to study the tissue concentrations of pentobarbital in rats injected with pentobarbital alone and in rats receiving both the barbiturate and the antihistamine.

The Effect of Antihistamines on the Rate of Disappearance of Pentobarbital from the Liver.—The results are shown in Fig. 2 with micrograms of pentobarbital per gram of liver plotted against time in minutes after anesthesia. A somewhat lower level of pentobarbital in the livers of the experimental animals would appear to be the logical result of a drain on any barbiturate reservoir by the brain. However, this difference was neither striking nor consistent throughout the duration of anesthesia. At the same time it must be kept in mind that the animals were assuredly not always on the same physiological plateau.

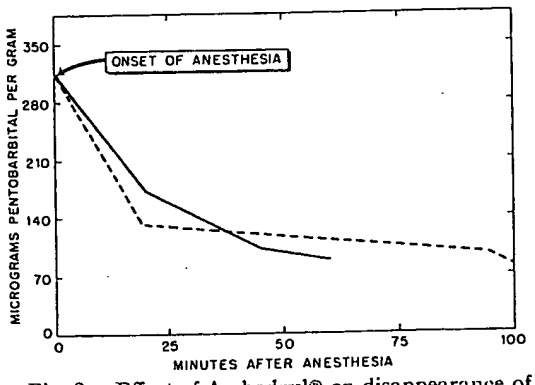

Fig. 2.—Effect of Ambodryl® on disappearance of pentobarbital from liver. ———, controls; – – – – Ambodryl®.

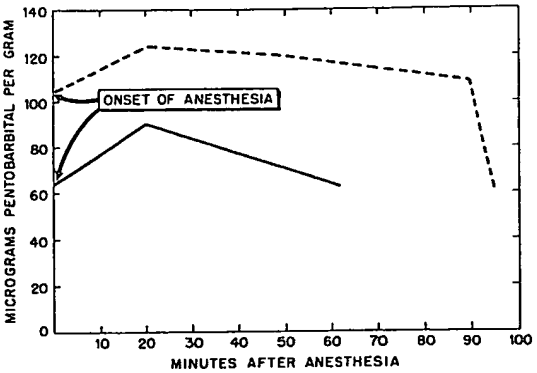

Fig. 3.—Pentobarbital levels in brain with and without Ambodryl®. ———, controls; – – – – Ambodryl®.

The Effect of the Antihistamines on the Concentration of Pentobarbital in the Brain.—In this experiment the entire brain was removed for analysis. The results are shown in Fig. 3. It would appear that the idea of increased rate of entrance of pentobarbital into the brain is borne out by these results. The concentration of pentobarbital into the brain is substantially higher at every time period from onset of anesthesia until the waking point at which time the concentrations were the same in both the control group and the experimental group. The mechanism of this enhanced entrance of the brain by pentobarbital would possibly have to be sought on an enzyme level.

Antihistamines and Enzymes.—Numerous reports have appeared indicting the antihistamines as disturbers of enzyme systems. The possibility of these actions being related to the central nervous system action of the antihistamines might well be kept in mind. Kreckowa (17) reported the antihistamines as effectively blocking the decarboxylation of pyruvate *in vitro* and also found the antihistamines increased the amount of pyruvic acid in the rabbit brain *in vivo*. Hubbard and Goldbaum (18) also found inhibition of pyruvate oxidation produced by pyribenzamine. Tickner (19) claims Benadryl®, Pyribenzamine®, Thephorin®, and Phenergan® block amine oxidase. Carlisle and Crescitelli (20) found Benadryl®, Pyribenzamine®, and Histadyl® were capable of selectively inhibiting the oxidation of *l*-glutamate while not interfering with glucose oxidation. All these facts are highly suggestive of some interference with carbohydrate metabolism of the brain and parallel some of the actions common to other hypnotics. While not a final answer to the mechanism of the depressant action of the antihistamines, they deserve further scrutiny.

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SUMMARY

1. Out of a total of twenty-two trials of comparing the sleeping times of animals injected with pentobarbital alone with sleeping times of animals injected with both pentobarbital and antihistamine only on three occasions did the sleeping times of the controls exceed those of the experimentals.

2. From the results of this investigation there is apparently no connection between incidence of clinical depression and the ability to modify the response to pentobarbital.

3. Ambodryl® injected thirty minutes prior to the administration of pentobarbital in rats produces a significantly higher concentration of pentobarbital in the grain than when the anesthetic is given alone.

4. Ambodryl® produces no significant effect upon the rate of disappearance of pentobarbital from the liver in rats.

5. The results of this work indicate that there must be a critical concentration of pentobarbital in the blood before the altered response produced by the antihistamines can be shown.

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Preliminary Studies Concerning the Lyophilized Water-Soluble Extracts of *Digitalis purpurea* L. and *Digitalis lutea* L.*

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A method for preparing lyophilized water-soluble extracts of *Digitalis purpurea* L. and *Digitalis lutea* L. has been described. The extracts which are instantaneously soluble in water, lend themselves readily to the extemporaneous preparations of infusions. Preliminary tests on the extracts stored approximately sixty days indicate that apparently the powders are stable and have potencies comparable with tinctures prepared from corresponding samples when equal concentrations are compared. Further studies concerning these extracts are now in progress.

DIGITALIS INFUSION has been a favorite form of digitalis medication among many physicians since its introduction to medicine in 1785 by Withering. While many investigators have found that the infusions of digitalis are prone to rapid decomposition, Weiss and Hatcher (1) found that their infusions represented more completely the potency of the digitalis leaves than did the tinctures.

Pomeroy and Heyl (2) studied the relative activity of the tinctures and infusions of equal concentrations and found infusions to be slightly less active than tinctures. This difference could be due to extraction factors, for Vartiainen and Yrjo (3) showed that one per cent infusions represented a greater percentage potency of the leaves than ten per cent infusions.

It was felt that if a stable water-soluble powder containing ingredients of the infusion could be made, some of the difficulties associated with the keeping qualities of the infusion might be reme-

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